

PHYSICAL PHENOMENA AND THE MICROGRAVITY RESPONSE

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INTRODUCTION

Whenever we have pursued simple physical descriptions of the inner workings of the cell we have discovered that nature was there long ago, genetically programming high-precision macromolecular machinery to assure the eternal persistence of a particular physical process, such as maintenance of the internal electrolytes of the cell by a collection of gates and pumps, maintenance of cell shape with not one or two, but at least three whole systems of cytoskeletal proteins, assuring the immortality of the genome itself through a complex system of repair enzymes we have barely begun to understand, etc. Very little about the cell is left to chance. But nature has never been given the opportunity to consider the maintenance of the living cell in the absence of net inertial acceleration and its consequences, such as hydrostatic pressure, buoyant flow, and sedimentation.

At the inception of space research some 30 years ago, there was concern in the U.S. and the Soviet Union about the effects of weightlessness on living things. It needed to be known in particular whether the absence of gravity had no effect or a catastrophic effect on biological systems under space flight conditions. It was easy to solve problems introduced by the space environment by the use of engineering to protect against the lack of an atmosphere and the presence of radiation, but engineering against weightlessness and its possible biological effects proved to be extremely difficult. Fortunately, early experiments indicated that the biological effects of low gravity were certainly not catastrophic, and the 84-day Skylab mission and substantially longer Soviet missions succeeded in the absence of a gravitational field. However, profound physiological changes were noted, and countermeasures are in use in modern manned space flights.

Current and future research is directed at the basic study of what we presume to be gravity dependent environmental responses. In other words, space flight conditions are being made available for basic science experiments.

Although we know of many biological phenomena affected by gravity, their connection to molecular and physical processes are poorly understood. In this sense, the effect of gravity is paradoxical because the cell is the basic structure of living things, and the organisms' properties depend upon cells. Yet it is much easier to think of gravity as acting on larger systems as cells are at the limit of size and mass which is influenced by the gravitational field in the presence of thermal motion.

Since the beginning of the orbital space flight era in 1957, scientific experiments on the effects of weightlessness on cells from all five living kingdoms have been performed (Edwards, 1969; Moskvitin & Vaulina, 1975; Saunders, 1971; Taylor, 1977; Young and Tremor, 1968 a,b). Opportunities to perform, let alone repeat, experiments in the microgravity environment of orbital space flight have been rare. Until recently there has been a tendency

to generalize on the basis of a small number of unrepeatable experiments. Early negative results (Montgomery et al., 1974) that tended to confirm negative predictions (Pollard, 1965) were at one time in danger of becoming dogma. The field of microgravity cell biology has suffered, not only from a paucity of reproducible data but from a constrained research paradigm in which an inadequate variety of physical phenomena has served as a resource for hypothesis testing.

It is the purpose of this article to review a broad range of gravity-dependent physical processes, including interactions among these processes and to indicate how they might apply at the dimensions of single cells.

But first, a few definitions may help guide investigations of gravitational effects at the single-cell level. While all cells on earth evolved in the presence of a 1-g field, some developed mechanisms to use this field (root and shoot gravitropism) while others developed countermeasures against its effect (muscle, cytoskeleton). The unnatural unloading of this force affects essential mechanism in the former case and fortuitous ones in the latter. Correspondingly, the former type of cell (plant, protozoa) responds to gravity, while the latter (animal) is affected by gravity. It is now possible to consider inertial acceleration as a continuous variable - all the way down to almost zero (10^{-6} - 10^{-4} x g), so while zero may be considered the origin of g as with any variable, the baseline value is $g = 1$ (or 9.8 m/sec^2). This somewhat inverted situation tempts one to study "the effect of microgravity" rather than to "perform g-unloading experiments."

A CORNUCOPIA OF GRAVITY-RELATED PHYSICAL PROCESSES

Inertial accelerations, including gravity, play a role in directly affecting the motion of masses and by contributing to motion when other forces are present. A few examples that apply to small particles and fluids are introduced.

1. Sedimentation

Stokes' sedimentation describes the constant velocity of a particle falling through a fluid, in which gravitational, buoyant, and viscous drag forces are balanced. Beginning with

$$F(\text{grav}) - F(\text{buoy}) - F(\text{drag}) = 0 \quad (1)$$

one finds for a sphere of radius a and density ρ that the "terminal velocity" is

$$v = 2(\rho - \rho_0) a^2 g / 9 \eta \quad (2)$$

where ρ_0 is the fluid density, and η is the fluid viscosity. It can be seen from equation (2) that sedimentation rate depends in a sensitive way on particle radius (squared) and density (from which fluid density is subtracted.)

2. Diffusion/Brownian motion

Einstein succeeded in describing diffusion as the consequence of a "random walk" executed by particles due to their thermal energy kT (k =Boltzmann's constant). The surprisingly simple result was

$$\langle x^2 \rangle = 2 D t \quad (3)$$

where $\langle x^2 \rangle$ = mean square distance travelled by a particle having diffusion coefficient D in time t . D can be derived from the thermal energy kT of a particle of radius a undergoing Brownian movement in a fluid with viscosity η :

$$D = k T / 4 \pi \eta a \quad (4)$$

These relationships give rise to Fick's laws of diffusion, in which the net unidirectional flux of particles is proportional to the gradient, dc/dx , of the particle concentration c .

Diffusion is not affected by gravity and occurs in its absence. However, diffusion and sedimentation velocities are sometimes similar, and their sum results in gradual settling; and under certain combinations of D , η , and dc/dx , the collective behavior of dissolved molecules and/or particles results in droplet (or zone) sedimentation.

3. Isothermal settling

If the temperature T does not change substantially over the height h of an ensemble of particles, then the mean kinetic energy kT of all particles is the same at all heights. The potential energy of a particle of mass m is usually expressed as mgh , but if the particles are subject to buoyancy in the fluid the potential energy becomes $V (\rho - \rho_0)gh$, if the particle volume is V . From the famous Boltzmann distribution rule the concentration of particles at height h will be established:

$$c(h) = c(0) \exp [-V(\rho - \rho_0) g h/kT]. \quad (5)$$

This means concentration is an exponential function of height under isothermal conditions and that large, dense particles with P.E. $\gg kT$ (from mammalian cells to marbles) will be concentrated at $h = 0$ and that small particles, such as certain organelles have values of V and ρ that lead to exponential distributions of $c(h)$ (Pollard, 1965).

4. Droplet sedimentation

The diffusion coefficients of small molecules are in the range of 10^{-6} to $10^{-5} \text{ cm}^2/\text{sec}$, of macromolecules 10^{-7} to 10^{-6} , and of whole cells and particles 10^{-12} to 10^{-9} . If a small zone, or droplet, of radius R contains n particles of radius a inside, whose diffusivity is much less than that of particles outside, then rapid diffusion of solutes in and slow diffusion of particles out of the droplet leads to a transient locally increased density of the droplet:

$$\rho_D = \rho_0 + \frac{a^3}{R^3} \eta (\rho - \rho_0). \quad (7)$$

If $\rho_D > \rho_0$ then the droplet falls down; if $\rho_D < \rho_0$ it is buoyed upward - the so-called Rayleigh-Taylor instability condition. Droplet sedimentation (or buoyancy) is a special case of a more general phenomenon—convection.

5. Convection

The sedimentation or buoyancy of fluid zones (large or small) often occurs due to thermal (temperature) gradients that cause lower zones to become less dense than zones above them. In a sense, motion of the type described by equation (2) follows, but, depending on the values of dimensionless ratios (Rayleigh number, Grasshof number), this motion can be spatially patterned (Bénard cells). In addition to thermal convection, solutal convection can occur when concentration gradients lead to dense solutions being found above less-dense solutions, even under isothermal conditions. Owing to the lack of good quantification of convection at small dimensions, we do not know whether or not convection inside a single cell is possible. It is quite apparent, however, that convective forces play a role in early post-nucleation events during the growth of crystals from solution (Kam et al., 1978).

6. Particle streaming

When solid particles or droplets of two densities are present, and when one particle type sediments downward while the other is buoyed upward, a traffic pattern is established whereby fine streams of alternating upward and downward fluid motion occur. Batchelor (1986) characterized this motion on the basis of a follow-the-leader paradigm which seems to be broadly applicable and represents yet another example of collective behavior of particles suspended in a fluid.

7. Flocculation and coalescence

Flocculation is the attachment of suspended particles or molecules to one another when Van der Waals interactions are not counteracted by electrostatic repulsion (colloid instability). Coalescence is the growth of liquid droplets or films within or on another immiscible liquid. These two chemically different phenomena have the same hydrodynamic outcome: the value of a^2 in equation (2) increases, thereby increasing v . Gravity often causes these phenomena to be non-linear, as the increase in a^2 increases the collision cross-section, thereby further enhancing the flocculation and coalescence phenomena. While coalescence is due to interfacial (surface) tension, flocculation is related to electrokinetic properties of molecules or particles. These two phenomena are independent of gravity and occur in its absence (Van Alstine et al., 1987); however, inertial unloading can profoundly affect the ability of these forces to act, and the rate at which they proceed.

8. Interfacial, or surface, tension

Surface tension is the force per unit length required to maintain a surface or an interface between 2 phases. Surface free energies for most liquids are $\gg kT$; when they are not "superfluidity" occurs. Although the cell's plasma membrane is composed primarily of lipid, the presence of transmembrane protein reduces its surface tension to less than 1% that of an oil-water interface (Davson-Danielli, 1951). Low-gravity research has provided a number of insights into interfacial behavior (Subramanian, 1986) because large drops and bubbles can be formed and manipulated. The water filling an entire drinking glass, for example, can, and does, form a perfect sphere. Do round cells sag on earth, and do flat cells become round in space flight (Pollard, 1974)? Certain animal eggs can be shown to "sag" when resting on a surface at specific stages; on the other hand all single-cell types studied in space to date have been makers of their own destiny. Their shapes have been determined by their cytoskeleton, the forces of which substantially exceed inertial and surface forces. Not all cell types are the same, however, and the polymerization bonds that shape the cell are weaker in some cell types than they are in others.

9. Particle electrokinetics

The surface charge density of suspended particles prevents their coagulation and leads to stability of lyophobic colloids. This stability is the backbone of such huge enterprises as paints and coatings, pulp and paper, sewage and fermentation, etc. The same charges, of course, lead to motion when such particles are suspended in an electric field. The particle surface has an electrokinetic ("zeta") potential, ζ , proportional to σ_e , its surface charge density - a few mV on stable particles, including cells in aqueous suspension. If the solution has dielectric constant ϵ , the electrophoretic velocity is

$$v = \frac{\zeta \epsilon}{6 \pi \eta} E \quad (8)$$

for small particles, such as molecules, whose radius of curvature is similar to that of a dissolved ion ("Debye-Hückel particles), and

$$v = \frac{\zeta \epsilon}{4 \pi \eta} E \quad (9)$$

for large ("Smoluchowski") particles, such as cells and organelles in an electric field, E .

10. Streaming potential

If a charged particle moves an electrical potential will be created, and this potential will impart motion to other charges in the environment, including dissolved ions. While the ζ potential of a stationary particle is only "felt" by

charges up to 7 Å or so away, an electric field spreads over greater distances when the particle moves. If a particle is caused to move by the acceleration of gravity (upward or downward) the strength (V/cm) of the electric field generated is

$$E = \frac{\zeta \epsilon (\rho - \rho_0) g}{3 \pi \eta \kappa} \quad (10)$$

where κ is the Debye-Hückel constant, measured in cm^{-1} and is directly proportional to the ionic strength of the surrounding medium. The force of this field is counter to the direction of motion of the particle, hence the name "counter streaming potential" also known as the "Dorn Effect." This potential could be as great as 20 mV.

11. Interacting fields

In reality, no force acts in the absence of other forces, and to some degree, from zero on upward, forces affect each other's actions. To deal with this fact, all types of flow (mass, charge, magnetic flux, etc.) are assumed to be non-independent, and transport relationships are described by a flow-and-field matrix. All flows J are caused by a field, generalized as $\Delta\mu$, in proportion to a coefficient L that relates them:

$$J = L \Delta\mu \quad (11)$$

For example, J might be the movement of mass falling through a specified area ($\text{kg m}^{-2} \text{sec}^{-1}$), $\Delta\mu$ would then be the inertial force field, in this case the acceleration of gravity, g over time Δt . L will convert the inertial coefficient (mass, in the simplest case) and the amount of material falling (concentration), or

$$J_m = N m g \Delta t \quad (12)$$

also familiar as Newton's 2nd law. Flow can be generalized on the basis of what is flowing, J_i , and the fields causing the flow, $\Delta\mu_j$; more than one type of field can cause more than one type of flow, so in general one has a matrix type of field:

$$J_1 = L_{11} \Delta\mu_1 + L_{12} \Delta\mu_2 + L_{13} \Delta\mu_3 \cdots$$

$$J_2 = L_{21} \Delta\mu_1 + L_{22} \Delta\mu_2 + L_{23} \Delta\mu_3 \cdots$$

$$J_3 = L_{31} \Delta\mu_1 + L_{32} \Delta\mu_2 + L_{33} \Delta\mu_3 \cdots$$

$$\text{or} \quad J_i = \sum_j L_{ij} \Delta\mu_j \quad (13)$$

This means, for example, electric fields can move charged masses and gravitational fields can move charges associated with mass. In this example (a falling charged particle) one can determine the downward mass flux, J_m , and the electric current $I = J_e$:

$$J_m = L_{11} \Delta\mu_g + L_{12} \Delta\mu_e \quad (14a)$$

$$J_e = L_{21} \Delta\mu_g + L_{22} \Delta\mu_e \quad (14b)$$

In most cases, L_{21} and L_{12} , the cross-term coefficients (the effect of gravity on a current and the effect of the electric field on sedimentation, respectively) are considered small compared to L_{11} and L_{22} . However, most physicists will point out that, at subcellular dimensions $\Delta\mu_e \gg \Delta\mu_g$, so it may not be possible to ignore cross terms in subcellular transport. In any case, solution of equations (14) at equilibrium leads to (Tobias et al., 1972):

$$J_m = \frac{8 \pi a^3 (\rho - \rho_0)^2 c g}{27 \eta} + \frac{\zeta \epsilon a^3 (\rho - \rho_0) c E}{3 \eta} \quad (15a)$$

$$J_e = \frac{\zeta \epsilon a^3 (\rho - \rho_0) g c}{3 \eta} + k E \quad (15b)$$

where k = specific conductivity and c = concentration. Each of these terms is recognizable, from the top, left to right, as Stokes sedimentation (equation (2)), Dorn-effect electrophoresis (equations (9) and (10)), streaming potential (equation (10)), and Ohm's law.

12. Work

Whole cells, and presumably their parts, are ultimately positioned vertically with respect to one another or some marker. In most cases, this means that each positioned object gained the potential energy associated with its vertical position h , above the place where it was born, by the performance of net work W , which is path-independent:

$$W = V (\rho - \rho_0) g h \quad (16)$$

SOME APPLICATIONS TO THE CELL

Phenomena to which the above-mentioned principles apply can be identified inside every cell and among cells. A few examples are considered here.

1. Sedimentation, Eukaryotic chromosome example

If the metaphase eukaryotic chromosome is considered as a compact object, as indicated in Figure 1, its sedimentation velocity can be estimated to be

around 2×10^{-7} cm/s -- similar to its rate of poleward migration during anaphase (Todd, 1977).

2. Sedimentation of organelles

If the same treatment is applied to selected organelles (those sufficiently large and dense to be worthy of consideration (Pollard, 1965; Fawcett, 1966; Tobias et al, 1972)), the approximate physical properties of each, given in Table 2, can be used to estimate the sedimentation velocities of each, also listed in Table 2. The final column in Table 2 indicates caution. Most of these organelles are anchored in place by cytoskeletal structures (in the case of chromosomes and the nucleus (Prescott et al., 1972; McNutt et al., 1973)) or embedded in internal membranes (in the case of mitochondria, plastids, and dictyosomes (Shen-Miller, 1972 a,b,c,d)), or both - see Table 3. Only the motions of otoliths and amyloplasts (statoliths) are known to be responsive to g and responsible for a measurable g-response (Audus, 1962, 1964; Gray and Edwards, 1971).

3. Isothermal settling of platelets

Human platelets stored in microgravity have a longer lifetime than their counterparts on the ground (Surgenor, 1987). Interactions that occur during settling are among the hypothetical causes of the short life span of the thrombocyte in vitro. While a certain amount of flocculation occurs during platelet storage, it is nevertheless reasonable to ask whether single-platelet suspensions actually settle. First, a Stokes' sedimentation velocity can be estimated as $0.01 \mu\text{m/s}$ (Table 4), which corresponds to about 1 diameter settling distance every five minutes. Brownian movement will lead to a final vertical distribution given by equation (5) in which the concentration of platelets, $c(h)$ is reduced by $1/e$ every $9 \mu\text{m}$ from the bottom of the container. It thus appears that, with or without flocculation, platelet settling is significant and cannot be dismissed as being unrelated to their short (a few days) lifespan in vitro.

4. Convection.

A study of early lattice formation in nucleating protein crystals (Kam et al., 1978) indicates that critical assembly processes occur at the submicron level. During lattice formation, the Gibbs free energy of crystallization is released to the immediate environment as heat, and solute is depleted near the lattice-forming surface. Both events lead to a local density reduction (Figure 2) with the potential for convection. The g-unloading of this process should, therefore, lead to higher quality crystal growth, which, evidently, it does (DeLucas et al., 1987; Bugg, 1987; Litke and John, 1982). Similarly, the formation of such self-assembled structures as microtubules (Weisenberg, et al, 1968) might be improved during g-unloading. Preliminary experiments by Moos et al (1988) indicate a more uniform length distribution of microtubules assembled during parabolic aircraft flight.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge introductions to this subject by C.A. Tobias and E.C. Pollard, stimulation to pursue it by D.R. Morrison, T.W. Halstead, E.K. Ray, and R.S. Bandurski, guidance from J.O. Kessler and A.H. Brown, and assistance with the preparation of this manuscript from J. Sibonga.

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TABLE I. HYDRODYNAMIC VALUES FOR A METAPHASE CHROMOSOME (SEE FIGURE 1) USED FOR APPLICATION TO EQUATION (2). CHROMOSOMES HAVE BEEN EXAMINED HYDRODYNAMICALLY IN ISOLATION (Burki et al., 1973; Schneider & Salzman, 1970), AND CYTOPLASMIC VISCOSITY HAS BEEN STUDIED BY PARAMAGNETIC RESONANCE (Keith & Snipes, 1974).

$$V = 2 \pi r^2 l = 25 \times 10^{-2} \text{ cm}^3$$

$$g = 980 \text{ cm/sec}^2$$

$$\rho - \rho_0 = 1.35 - 1.04 = 0.31 \text{ g/cm}^3$$

$$a = (3V/4\pi)^{1/3} = 2.1 \times 10^{-4} \text{ cm}$$

$$h = 5 \pm 2 \text{ dyn-sec/cm}^2$$

$$v = 2 \times 10^{-7} \text{ cm/sec}$$

TABLE 2. PHYSICAL PROPERTIES OF ORGANELLES USED TO CALCULATE STOKES' SEDIMENTATION VELOCITIES

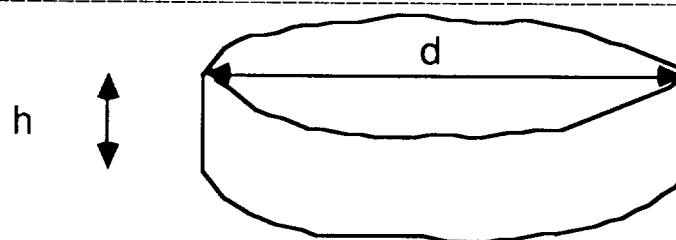
<u>ORGANELLE</u>	<u>VOL</u> (μm^3)	ρ (g/cm^3)	$\rho - \rho_0$	v (cm/sec)	t (sec)	x (μm)	<u>FEATURE</u>
MITOCHONDRION	2-100	1.1	0.01-.02	$0.1-4 \times 10^{-8}$	10^3	0.1	Convolved, large structure
NUCLEOLUS	10-20	1.4	0.3	2×10^{-7}	10^4	20	Suspended by chromatin
CHROMOSOME	5-50	1.35	0.3	2×10^{-7}	10^3	2	Suspended by μ tubules
AMYLOPLAST	100	1.5	0.4	1×10^{-6}	$\leq 10^3$	10	Real free particle
OTOLITH	1000	2.0	0.8	$\geq 1 \times 10^{-5}$	1	0.1	Known to react
DICTYSOME	100	1.2	0.15	$\geq 3 \times 10^{-7}$	10^3	2	Internal membrane structure

Some data derived from Fawcett (1966).

TABLE 3. ORGANELLES THAT COULD SEDIMENT

<u>ORGANELLE</u>	<u>a, MICRONS</u>	<u>ORIGIN OF TENSILE FORCE</u>
NUCLEUS	5	10 NM FILAMENTS
NUCLEOLUS	1	CHROMATIN
CHROMOSOME	2	MICROTUBULES
CILIUM	4 - 10	MICROTUBULES
DICTYOSOME	2 - 6	MICROTUBULES

TABLE 4. STOKES' PARAMETERS FOR THROMBOCYTES IN PLASMA AND CALCULATION OF THEIR SEDIMENTATION VELOCITY



$$h = 0.5 \mu\text{m}$$

$$d = 3.0 \mu\text{m}$$

Equivalent Stokes' radius = $0.94 \mu\text{m}$ from $a = (3V/4\pi)^{1/3}$

Density from Geigy tables

$$\rho \text{ (platelet)} = 1.045 \text{ g/cm}^3$$

$$\rho \text{ (plasma)} = 1.0269 \text{ g/cm}^3$$

$$\eta \text{ (plasma)} = 1.10 \text{ cp} = 0.011 \text{ g/sec-cm}$$

Velocity

$$v = \frac{2(\rho - \rho_0) a^2 g}{9 \eta} = 0.01 \mu\text{m/sec} \approx 1 \text{ diameter/ } 5 \text{ min}$$

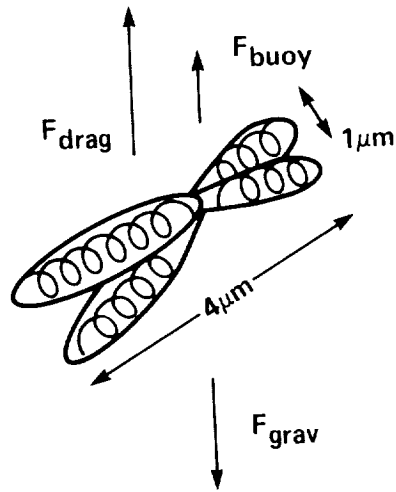


Figure 1. Balance of forces and dimensions of a metaphase chromosome sedimenting in free solution.

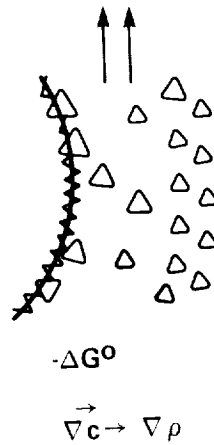


Figure 2. Events at the surface of a growing particle (crystal) that lead to fluid instability. The free energy of binding (or lattice formation) is released to the immediate fluid environment thereby raising its temperature and decreasing its density. At rapid growth rate, adsorption is more rapid than diffusion and solute concentration drops thereby decreasing the solution density. Both phenomena could lead to buoyancy of fluid at the growing surface.